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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

HOGAN *et al.*

Serial No. 08/454,529

Filed: May 30, 1995

For: METHODS FOR DETERMINING  
THE PRESENCE OF NON-VIRAL  
ORGANISMS IN A SAMPLE

) Group Art Unit: 1631

) Examiner: Marschel, A.

) Atty. Docket No. GP004-16.DV4

) VIA FEDERAL EXPRESS

#37  
Plunkett  
7/8/02

**DECLARATION UNDER 37 C.F.R. § 1.131**

Examiner Ardin H. Marschel  
Art Unit 1631  
United States Patent and Trademark Office  
7<sup>th</sup> Floor Receptionist  
1911 South Clark Place  
Crystal Mall One  
Arlington, Virginia 22202

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JUL 3 2002

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Dear Examiner Marschel:

We, James J. Hogan, Richard D. Smith, JoAnn Kop Dileanis and Sherrol H. McDonough, co-inventors of the above-identified patent application, upon information and belief hereby declare as follows:

1. Prior to December 1986, we conceived of and reduced to practice in the United States an oligonucleotide probe selected to distinguish *Chlamydia trachomatis* from a closely related non-target species belonging to the *Chlamydia* genus. Evidence of this prior conception and reduction to practice was recorded in the laboratory notebooks of Ame L. Holden, Mohammad R. Majlessi, Mary E. Harper and Paula Roeder, all employees of the assignee of the instant application at the time of the claimed invention. Ame Holden was working as a Research Associate under the supervision of Sherrol McDonough, Mary Harper was a Staff Scientist in Product Development, Paula Roeder was a Research Scientist in Product Development, and Mohammad Majlessi was a

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Research Associate responsible for oligonucleotide synthesis. The relevant pages from the laboratory notebooks of Ame Holden, Mohammad Majlessi, Mary Harper and Paula Roeder are attached hereto as Exhibits A, B, C and D, respectively. Although the dates on these notebook pages have been redacted, as well as information which is not relevant to establishing prior conception and reduction to practice, the activities set forth therein were performed exclusively in the United States and were completed prior to December 1986.

2. Exhibit A shows a comparison of the corresponding base regions of 16S rRNA from closely related *Chlamydia trachomatis* and *Chlamydia psittaci* organisms that was performed by Ame Holden under Sherrol McDonough's supervision prior to the critical date. See Exhibit A, Book No. 329, p. 32 (thymine is substituted for uracil in the sequences being compared). From this comparison, it was determined that there are a total of twelve base differences between the sequences of these two *Chlamydia* organisms in this region.

3. Based on this sequence information, Ame Holden, under the supervision of Sherrol McDonough, diligently proceeded to have an oligonucleotide probe synthesized which was fully complementary to the *Chlamydia trachomatis* sequence identified as "ChtA0176" in Ame Holden's notebook. See Exhibit A. The synthesis name given to this sequence by Mohammad Majlessi was "ChtA176". See Exhibit B, Book No. 320, page 39. As can be seen from Exhibit B, oligonucleotide probes having this sequence were synthesized by Mohammad Majlessi on a G column using an ABI 380A DNA Synthesizer. The coupling efficiency was analyzed using a trityl-off procedure. See Exhibit B at page 40. The synthesized probes were then purified on a polyacrylamide gel, as shown in the autoradiograph at page 39 of Exhibit B. Optical density measurements on the purified sample indicated the presence of nucleic acid, and the autoradiograph depicted at page 40 of Exhibit B confirms that the synthesized sequence was 33 nucleotides in length.

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4. The sequence of the ChtA0176 probe conceived of and reduced to practice by us corresponds to bases 176-208 of *E. coli* 16S rRNA.

5. Mary Harper diligently labeled the ChtA0176 probe synthesized by Mohammad Majlessi with <sup>32</sup>P by kinasing. *See* Exhibit C, Book No. 391, pages 25-26.

6. The kinased ChtA0176 probe was obtained by Paula Roeder, who diligently used the probe to conduct a specificity study. *See* Exhibit D, Book No. 405, pages 35 and 39-42. The ChtA0176 probe was provided to a solution containing a DIBSS diluent and a second, iodinated pan-bacterial probe (Probe 1082) which was included to demonstrate the presence of bacterial RNA in the hybridization mixtures. Eleven duplicate hybridization mixtures were prepared in scintillation vials, each vial receiving the same amount of the ChtA0176 probe. *See* Exhibit D at page 39. Except for a negative control, each hybridization mixture set included one of the following: (i) a lysate from one of five different *Chlamydia trachomatis* serotypes; (ii) a lysate from one of three different *Chlamydia psittaci* serotypes; (iii) RNA from one *Chlamydia trachomatis* serotype; or (iv) RNA from *Escherichia coli*. *See* Exhibit D at pages 39 and 42. The hybridization mixtures were incubated for one hour at 64°C. *See* Exhibit D at page 40. After incubating the hybridization mixtures, 4.5 ml separation solution was added to each vial and the vials were incubated for five minutes at 64°C. *See* Exhibit D at pages 35 and 40. The separation solution contained hydroxyapatite (HA) and was used to separate hybridized probe from unhybridized probe in the hybridization mixtures. Hybrids bound to HA were separated from unbound probe by centrifuging the vials for a period of time sufficient to pellet the HA and decanting the resulting supernatants into separate, corresponding scintillation vials. The HA-containing vials were then washed with 5.0 ml of a 0.14 M phosphate buffered solution, vortexed, incubated for another five minutes at 64°C and centrifuged to pellet HA in the vials. The supernatants generated by this second centrifugation were also decanted into the corresponding vials prepared after the first centrifugation.

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7. The  $^{32}\text{P}$  level in each vial was measured for two minutes in a scintillation counter (referred to as "HAL" at page 40 of Exhibit D), and the results are presented at page 41 of Exhibit D. Each numbered pair in the results (*e.g.*, 1 and 2) represents a duplicate set of hybridization mixtures. And for each numbered pair, the value associated with "BP" is a measure of the counts per minute in the vials containing hybridized or bound probe (*i.e.*, the HA-containing vials) and the value associated with "FP" is a measure of the counts per minute in the vials containing unhybridized or free probe. The percentage of bound probe was calculated by dividing the BP counts per minute by the total counts per minute (BP + FP) for each hybridization mixture. The percentage of bound probe was averaged for each duplicate set of hybridization mixtures, and the results are set forth at page 42 of Exhibit D. The results show that the ChtA0176 probe was able to detectably hybridize to RNA belonging to all of the *Chlamydia trachomatis* serotypes tested. The percentage of bound probe results for the negative controls and the hybridization mixtures containing nucleic acid from a *Chlamydia psittaci* serotype or *Escherichia coli* indicate that the ChtA0176 probe is specific for RNA belonging to *Chlamydia trachomatis*.

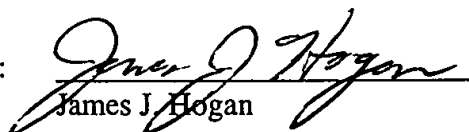
8. To demonstrate that bacterial RNA was present in the sample-containing hybridization mixtures and not in the negative controls, 0.09 ml probe solution and 5.0 ml CytoScint was added to a scintillation vial containing HA in an amount equivalent to that contained in the vials described in paragraph 6 above. The counts per minute in this vial were determined in the scintillation counter and used as the total counts per minute for the pan-bacterial probe. Each of the HA-containing vials described in paragraph 6 above then received 5.0 ml CytoScint and was counted for two minutes in the scintillation counter. For each duplicate set of hybridization mixtures, the counts per minute were averaged and divided by the total counts per minute to arrive at a percentage of hybridization for the pan-bacterial probe. These values are set forth at page 42 of Exhibit D and evidence that there was bacterial RNA in each of the vials except the negative controls.

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We hereby declare that all statements made herein of our own knowledge are true, and that statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application and any patent issuing therefrom.

Date: 6/24/02

By:   
James J. Hogan

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Richard D. Smith

Date: \_\_\_\_\_

By: \_\_\_\_\_  
JoAnn Kop Dileanis  
(formerly JoAnn Kop)

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Sherrol H. McDonough

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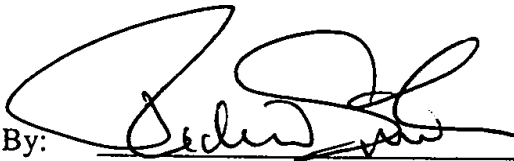
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Date: \_\_\_\_\_

By: \_\_\_\_\_  
James J. Hogan

Date: June 25, 2002

By:   
Richard D. Smith

Date: \_\_\_\_\_

By: \_\_\_\_\_  
JoAnn Kop Dileanis  
(formerly JoAnn Kop)

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Sherrol H. McDonough

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Date: \_\_\_\_\_

By: \_\_\_\_\_  
Sherrol H. McDonough

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By: \_\_\_\_\_  
James J. Hogan

Date: \_\_\_\_\_

By: \_\_\_\_\_  
Richard D. Smith

Date: \_\_\_\_\_

By: \_\_\_\_\_  
JoAnn Kop Dileanis  
(formerly JoAnn Kop)

Date: 6/27/02

By: Sherrol H. McDonough  
Sherrol H. McDonough